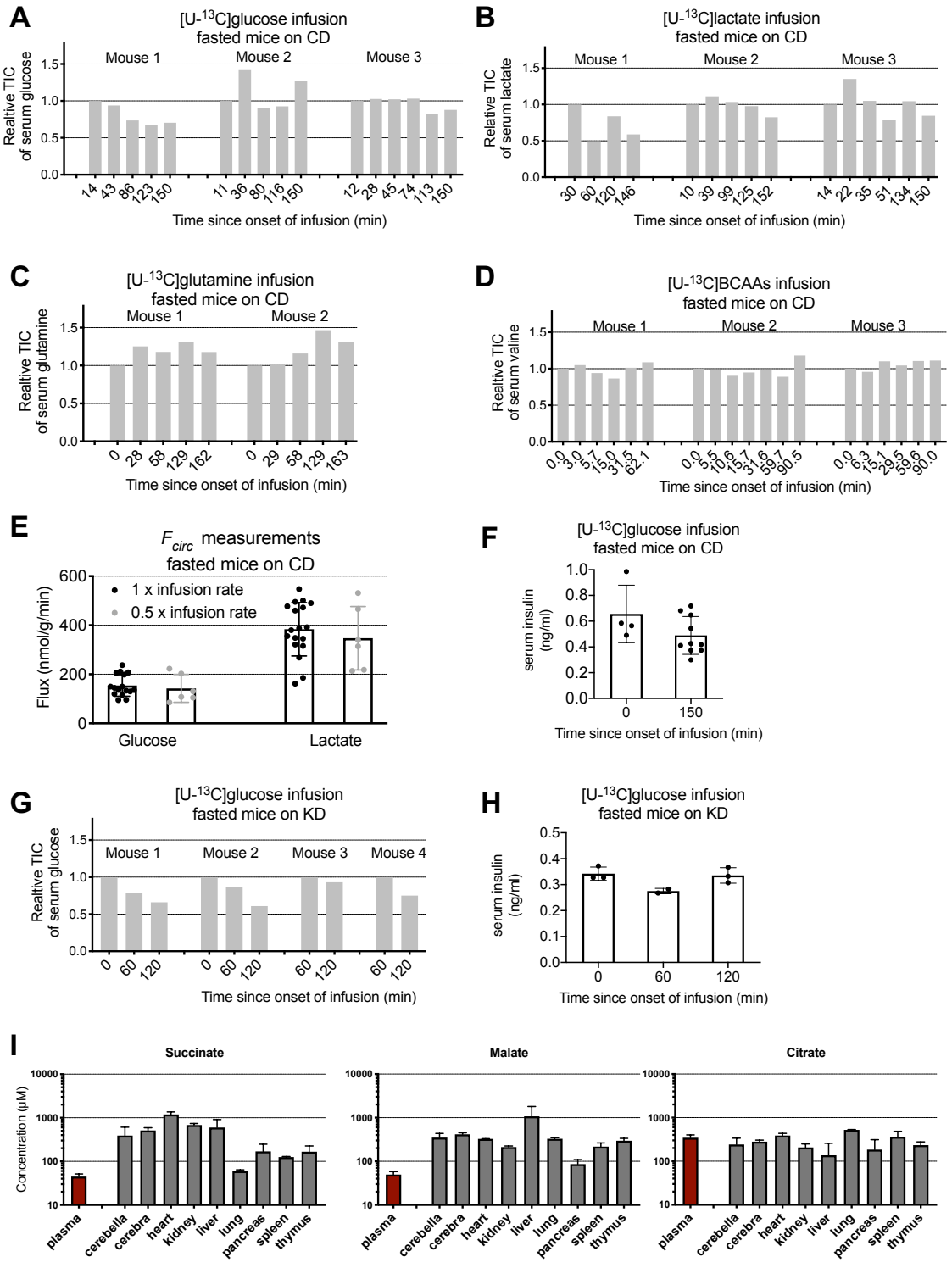

Figure S1. Levels of serum metabolites and insulin during infusions, in mice on either CD or KD. Related to Figure 1.

(A) Serum glucose level during the glucose infusion in fasted mice on CD.

(B) Serum lactate level during the lactate infusion in fasted mice on CD.

(C) Serum glutamine level during the glutamine infusion in fasted mice on CD.

(D) Serum valine level during the infusion of the BCAA combo in fasted mice on CD.
(E) F_{circ} values of glucose and lactate determined using different infusion rates. Bar graphs show mean ± SD
(F) Serum insulin level during the glucose infusion in fasted mice on CD.
(G) Serum glucose level during the glucose infusion in fasted mice on KD.
(H) Serum insulin level during the glucose infusion in fasted mice on KD.
(I) Concentrations of succinate and malate are generally higher in tissues than in plasma, while citrate has similar concentration between tissues and in plasma. Data are from the Mouse Multiple tissue Metabolome Database (http://mmdb.iab.keio.ac.jp). Values are mean ± SD (n=2 mice). Note that Y-axis is logarithmic scale.

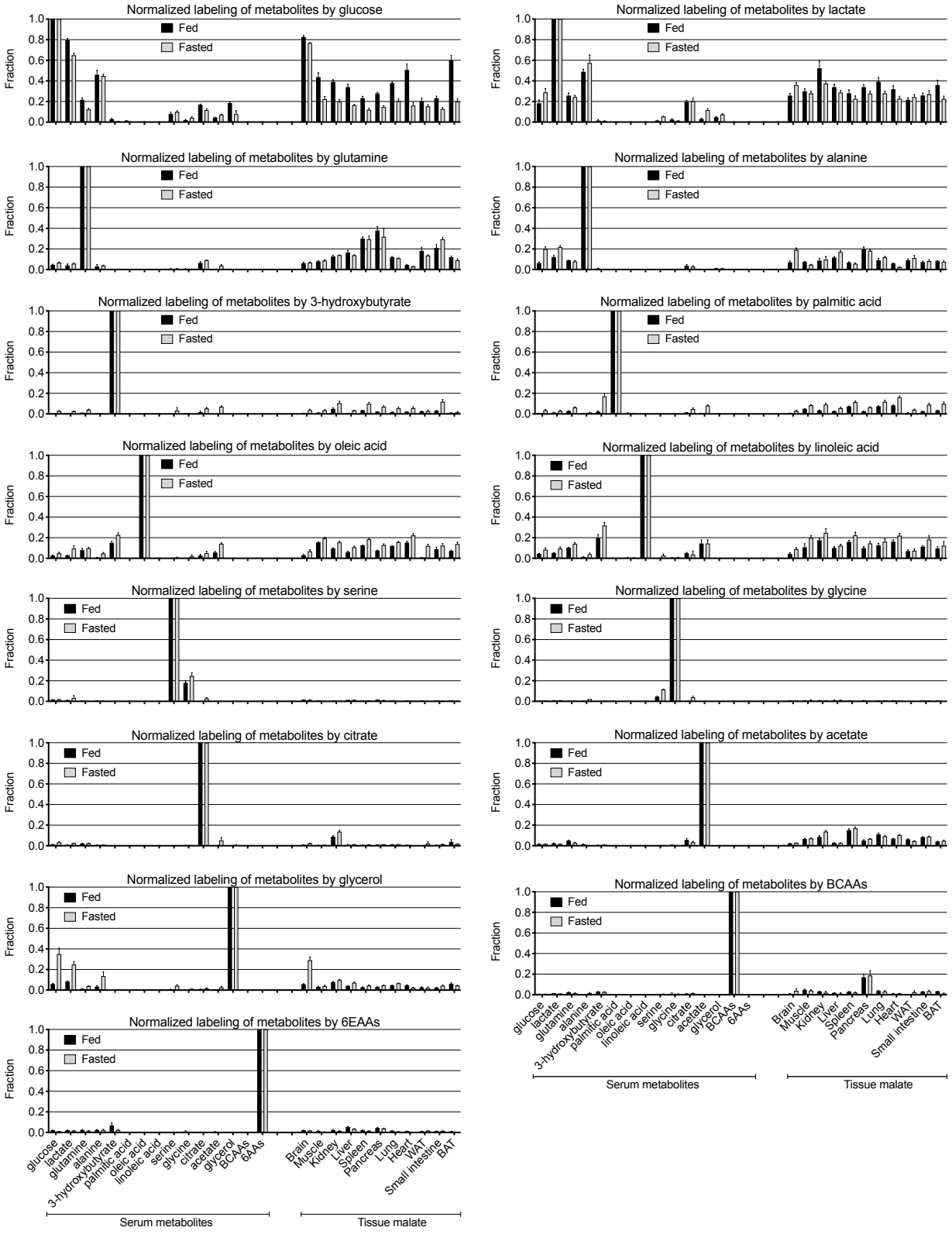

Figure S2: Normalized labeling data of metabolites by each of the 15 nutrients for mice on the carbohydrate diet. Data and number of repeats are in Table S3. Related to Figure 2 and Figure 3.

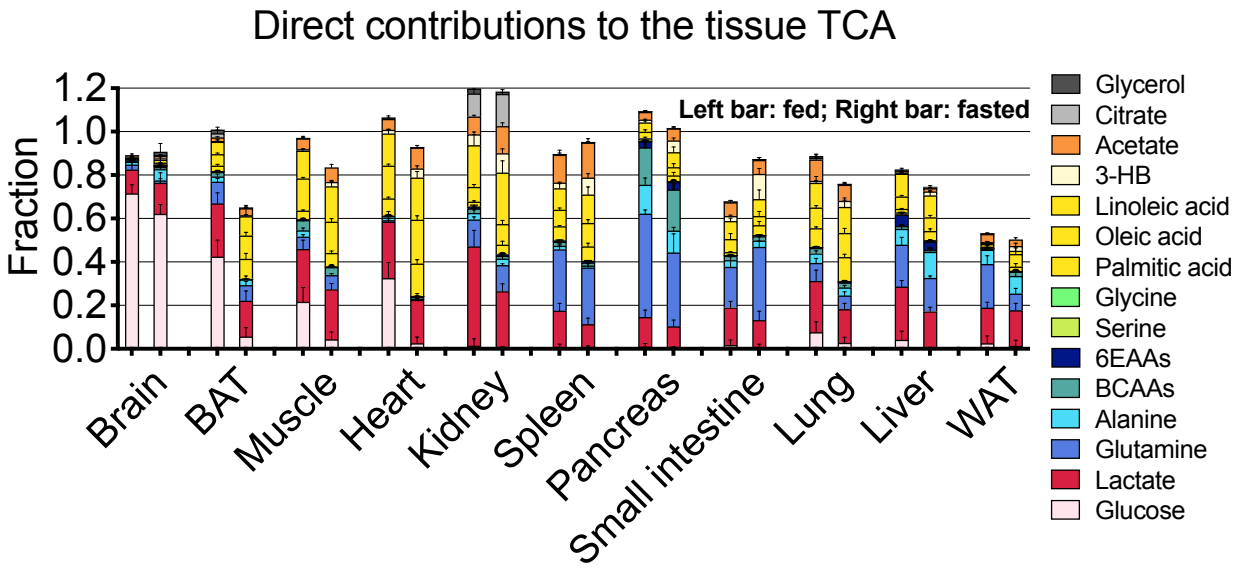

Figure S3: Direct TCA contributions using tissue succinate labeling measurements for 8-h fasted mice and 3-h refed mice on carbohydrate diet. Related to Figure 4.

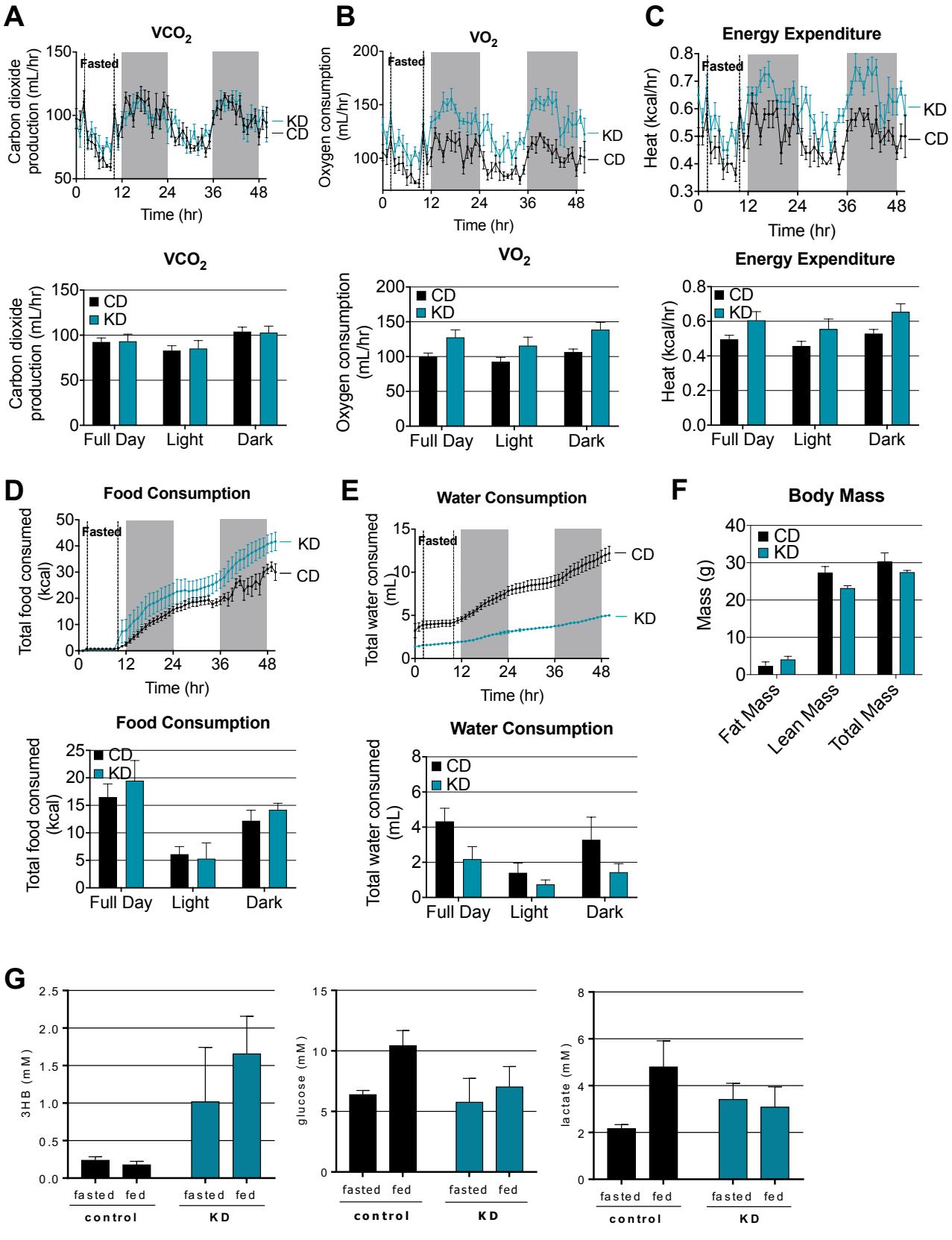
A
VCO2
Carbon dioxide production (mL/hr)
Fasted
KD
CD
Time (hr)
VCO2
CD
KD
Full Day
Light
Dark
B
VO2
Oxygen consumption (mL/hr)
Fasted
KD
CD
Time (hr)
VO2
CD
KD
Full Day
Light
Dark
C
Energy Expenditure
Heat (kcal/hr)
Fasted
KD
CD
Time (hr)
Energy Expenditure
CD
KD
Full Day
Light
Dark
D
Food Consumption
Total food consumed (kcal)
Fasted
KD
CD
Time (hr)
Food Consumption
CD
KD
Total food consumed (kcal)
Full Day
Light
Dark
E
Water Consumption
Total water consumed (mL)
Fasted
CD
KD
Time (hr)
Water Consumption
CD
KD
Total water consumed (mL)
Full Day
Light
Dark
F
Body Mass
CD
KD
Mass (g)
Fat Mass
Lean Mass
Total Mass
G
3HB (mM)
fasted
fed
control
KD
glucose (mM)
lactate (mM)

Figure S4. Metabolic and physical parameters of mice fed carbohydrate diet (CD; N=5) and ketogenic diet (KD; N=4). Bar graphs show mean ± SD, excluding the fasting period. Related to Figure 5.

(A) Oxygen consumption. Shaded areas indicate the dark periods. Mice were fasted for 8 h in the first light period.
(B) Carbon dioxide consumption.
(C) Energy expenditure.
(D) Food consumption.
(E) Water consumption.
(F) Body composition.
(G) Serum concentrations of 3-hydroxybutyrate, glucose and lactate.

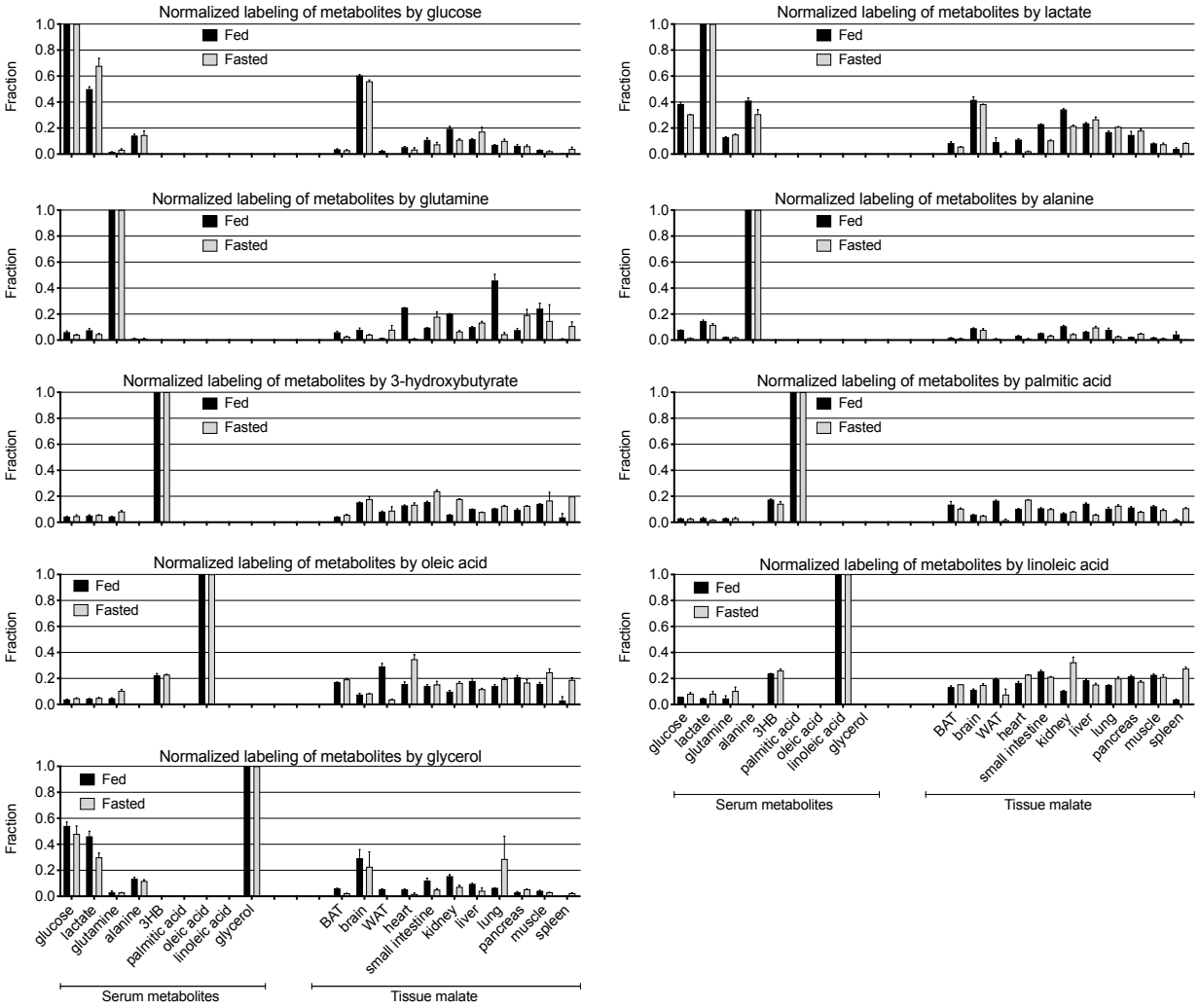

Figure S5. Normalized labeling data of metabolites by each of the 9 nutrients for mice on the ketogenic diet. Data and number of repeats are in Table S6. Related to Figure 5 and Figure 6.

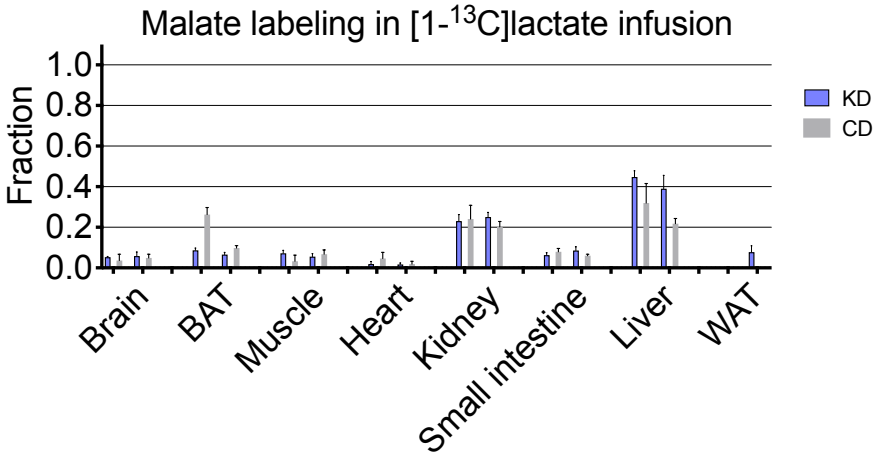

Figure S6. Tissue malate labeling by [1-^{13}C]lactate for mice on either CD or KD. For each tissue, the left two bars are for fed mice and the right two bars for fasted mice. Related to Figure 6.

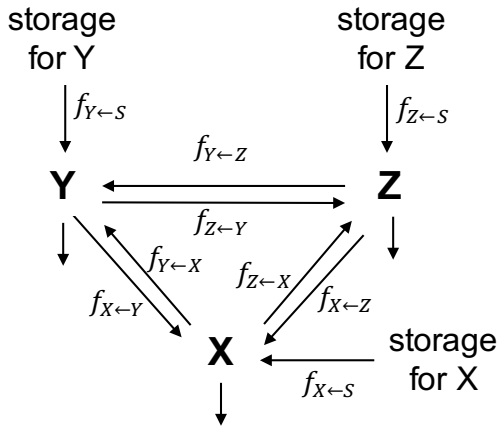

Figure S7. A simple case illustrating the inter-conversion between 3 circulating nutrients. The f represents the fractional direct contribution from one nutrient to another. Related to STAR Methods.

Methods S1: Quantifying fluxes between circulating nutrients. Related to STAR Methods.

In this note, we explain the procedure for calculating fluxes between circulating nutrients. The input data for this calculation are (i) an *n* x *n* matrix *M,* that reflects the extent to which infusion of any nutrient *i* (of *n* total nutrients of interest) labels every other circulating nutrient *j* ("the inter-labeling matrix") (note that generation of this matrix requires *n* isotope tracer infusion experiments) and (ii) the carbon-atom circulatory turnover flux F_{circ}^{atom} for each circulating nutrient *i* (defined as $F_{circ}^{atom} = C_i \frac{R_i(1-L_i)}{L_i}$ where C_i is the number of carbon atoms in one molecule of nutrient *i*, L_i is the fraction of labeled carbon atoms in the nutrient *i,* and R_i is the infusion rate of uniformly ^{13}C-labeled *i*). The procedure first uses *M* to calculate the direct contributions to each nutrient *i* from all other circulating nutrients *j*, creating a new n x n matrix *N* whose entries N_{ij} reflect the (fractional) direct contributions of circulating nutrient *j* to circulating nutrient *i*. It then utilizes the matrix *N* and the F_{circ}^{atom} to calculate the direct contributing fluxes from any circulating nutrient to any other circulating nutrient, resulting in a complete determination of the inter-converting fluxes between circulating nutrients.

Calculating the direct contributions between circulating nutrients. As detailed in the main text, by constructing a set of linear equations from the inter-labeling matrix *M*, we can calculate the direct contribution to any one circulating nutrient from the other circulating nutrients. The direct contribution from nutrient *j* to nutrient *i* is defined as the fraction of *i* that comes directly from *j*, e.g., 0.7 (or 70%) of circulating lactate comes directly from circulating glucose. The results can be organized as a second $n \times n$ matrix (denoted as *N*), with the non-diagonal entry N_{ij} representing the direct contribution from nutrient *j* to nutrient *i*, or

$$N_{ij} = f_{i \leftarrow j} \; for \; i \neq j \qquad [1]$$

For the diagonal entries in *N*, we define

$$N_{ii} = 1 - \sum_{j=1, j \neq i}^{n} N_{ij} \qquad [2]$$

which represents the direct contribution from sources other than circulating nutrients (i.e., nutrient storages). We thus denote the diagonal entries

$$N_{ii} = f_{i \leftarrow S} \qquad [3]$$

Calculating the direct contributing fluxes to a circulating nutrient. The matrix *N* contains the direct contribution values as fractions. To obtain the direct contribution in flux units (e.g., nmol carbon/min/g), we utilize the measured carbon-atom circulatory turnover flux values F_{circ}^{atom}. The calculation is more complex than just multiplying the direct contributions by F_{circ}^{atom}, however, because only unlabeled inputs to a metabolite contribute to F_{circ}^{atom}. The total flux also includes metabolic cycles where an infused labeled metabolite may be regenerated in labeled form from its own products. This requires multiplying F_{circ}^{atom} by an adjustment factor $c \geq 1$. For illustration, we use a simple case where there are only 3 circulating nutrients (Figure S7).

We first focus on one nutrient X and calculate the direct contributing fluxes to it: the flux from Y to X ($J_{X\leftarrow Y}$), the flux from Z to X ($J_{X\leftarrow Z}$), and the flux from any unmeasured, unlabeled inputs to X ($J_{X\leftarrow S}$) (the most important unlabeled inputs are diet and storage polymers). We denote J_X as the total flux that goes through X, meaning it is equal to the sum of the incoming fluxes to X (which at steady-state is also equal to the sum of the outgoing fluxes from X). Mathematically,

$$J_X = J_{X\leftarrow Y} + J_{X\leftarrow Z} + J_{X\leftarrow S} \quad [4]$$

$$J_{X\leftarrow Y} = J_X \cdot f_{X\leftarrow Y} \quad [5]$$

$$J_{X\leftarrow Z} = J_X \cdot f_{X\leftarrow Z} \quad [6]$$

$$J_{X\leftarrow S} = J_X \cdot f_{X\leftarrow S} \quad [7]$$

To calculate J_X, we begin by expressing F_{circX}^{atom} in terms of the fluxes on the network shown in Fig. 1. F_{circX}^{atom} reflects flux of unlabeled carbon into X. Such unlabeled carbon can come from multiple sources.

Path 1: external input to X (e.g. storage polymer, food)

$$f_{X\leftarrow S} \cdot J_X \quad [8]$$

Path 2: external input to Y → X

$$f_{Y\leftarrow S} \cdot J_Y \cdot \frac{f_{X\leftarrow Y} \cdot J_X}{J_Y} = f_{Y\leftarrow S} \cdot f_{X\leftarrow Y} \cdot J_X \quad [9]$$

where J_Y is the total flux through the nutrient Y.

Path 3: external input to Y → Z → X

$$f_{Y\leftarrow S} \cdot J_Y \cdot \frac{f_{Z\leftarrow Y} \cdot J_Z}{J_Y} \cdot \frac{f_{X\leftarrow Z} \cdot J_X}{J_Z} = f_{Y\leftarrow S} \cdot f_{Z\leftarrow Y} \cdot f_{X\leftarrow Z} \cdot J_X \quad [10]$$

where J_Z is the total flux through the nutrient Z.

Path 4: external input to Z → X

$$f_{Z\leftarrow S} \cdot J_Z \cdot \frac{f_{X\leftarrow Z} \cdot J_X}{J_Z} = f_{Z\leftarrow S} \cdot f_{X\leftarrow Z} \cdot J_X \quad [11]$$

Path 5: external input to Z → Y → X

$$f_{Z\leftarrow S} \cdot J_Z \cdot \frac{f_{Y\leftarrow Z} \cdot J_Y}{J_Z} \cdot \frac{f_{X\leftarrow Y} \cdot J_X}{J_Y} = f_{Z\leftarrow S} \cdot f_{Y\leftarrow Z} \cdot f_{X\leftarrow Y} \cdot J_X \quad [12]$$

Together the 5 fluxes above give rise to F_{circX}^{atom}, or

$$F_{circX}^{atom} = (f_{X\leftarrow S} + f_{Y\leftarrow S} \cdot f_{X\leftarrow Y} + f_{Y\leftarrow S} \cdot f_{Z\leftarrow Y} \cdot f_{X\leftarrow Z} + f_{Z\leftarrow S} \cdot f_{X\leftarrow Z} + f_{Z\leftarrow S} \cdot f_{Y\leftarrow Z} \cdot f_{X\leftarrow Y}) \cdot J_X \quad [13]$$

Denoting the coefficient between F_{circX}^{atom} and J_X as c_X, we rewrite Eqn. [13] as

$$J_X = c_X F_{circX}^{atom} \quad [14]$$

where

$$c_X = 1/(f_{X\leftarrow S} + f_{Y\leftarrow S} \cdot f_{X\leftarrow Y} + f_{Y\leftarrow S} \cdot f_{Z\leftarrow Y} \cdot f_{X\leftarrow Z} + f_{Z\leftarrow S} \cdot f_{X\leftarrow Z} + f_{Z\leftarrow S} \cdot f_{Y\leftarrow Z} \cdot f_{X\leftarrow Y}) \quad [15]$$

Thus, with Eqns. [14-15] and the direct contribution matrix *N*, we can calculate J_X for a network of 3 metabolites. Note that this equation can be generalized to any number of metabolites. Eqns. [5-7] then give the individual direct contributing fluxes to X.